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FUNGI—SOURCES OF FOOD, FUEL, AND BIOCHEMICALS[1]

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The occasion of the 50th Anniversary of the Mycological Society of America has prompted many of us to take stock of accomplishments made through the use of fungi. The results of these musings were the numerous summary presentations given at the 1981 annual meeting celebrating the Society's Golden Anniversary. This paper, derived from one of those presentations, aims briefly to provide examples of the involvement of fungi in the production of food, fuel, and biochemicals. Others in this review series will discuss secondary metabolites including fungus-synthesized antibiotics and such other areas as mushroom culture.

Mankind has been in the fermentation business for at least 8000 yr. The ancient peoples of Sumeria, Babylon, and Ur documented beer making in their artwork, and somewhat later (*ca.* 4000 B.C.), the Egyptians depicted the baking of leavened bread. Although these processes were used worldwide, it was little more than 100 years ago that independent studies reported in 1837 by Cagniard de la Tour, Schwann, and Kützing showed yeasts to be responsible for fermentation. Fermentations involving fungi include traditional foods and beverages, biochemicals, waste disposal, and more recently, production of liquid fuels. The myriad of products and processes allows detailing of only a few in this review.

FERMENTED FOODS

The subject of traditional fermented foods has been dealt with extensively by Hesseltine (**1965**) and Wang and Hesseltine (**1979**). As they have documented, there are a large number of such foods, and the fermentations involve bacteria, yeasts, and molds. The manufacture of soy sauce requires numerous steps and several different microorganisms; for this reason, it provides a good example of fermented food manufacture. Soy sauce is widely used throughout East Asia as a seasoning for foods, and it is growing in popularity among non-Orientals. The volume of soy sauce produced annually in Japan now amounts to one billion liters.

Soy sauce is made by fermenting soybeans and wheat with a mixture of molds, yeasts, and bacteria (FIG. 1). The fermentation is essentially an enzymatic hydrolysis of proteins, carbohydrates, and other soybean and wheat constituents to such low molecular weight compounds as peptides, amino acids, sugars, alcohols, and organic acids. Soybeans are prepared for fermentation by first soaking in water overnight and then steaming at 10 lb/in^2 for several hours (Wang and Hesseltine, **1979**). Soaking and steaming modifies the soy protein so that it is more susceptible to microbial enzymes. In recent years, defatted soybean meal and flakes have largely supplanted use of whole soybeans because there is then no problem of oil removal. The wheat, which provides an abundant carbon source for the mold

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[2] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

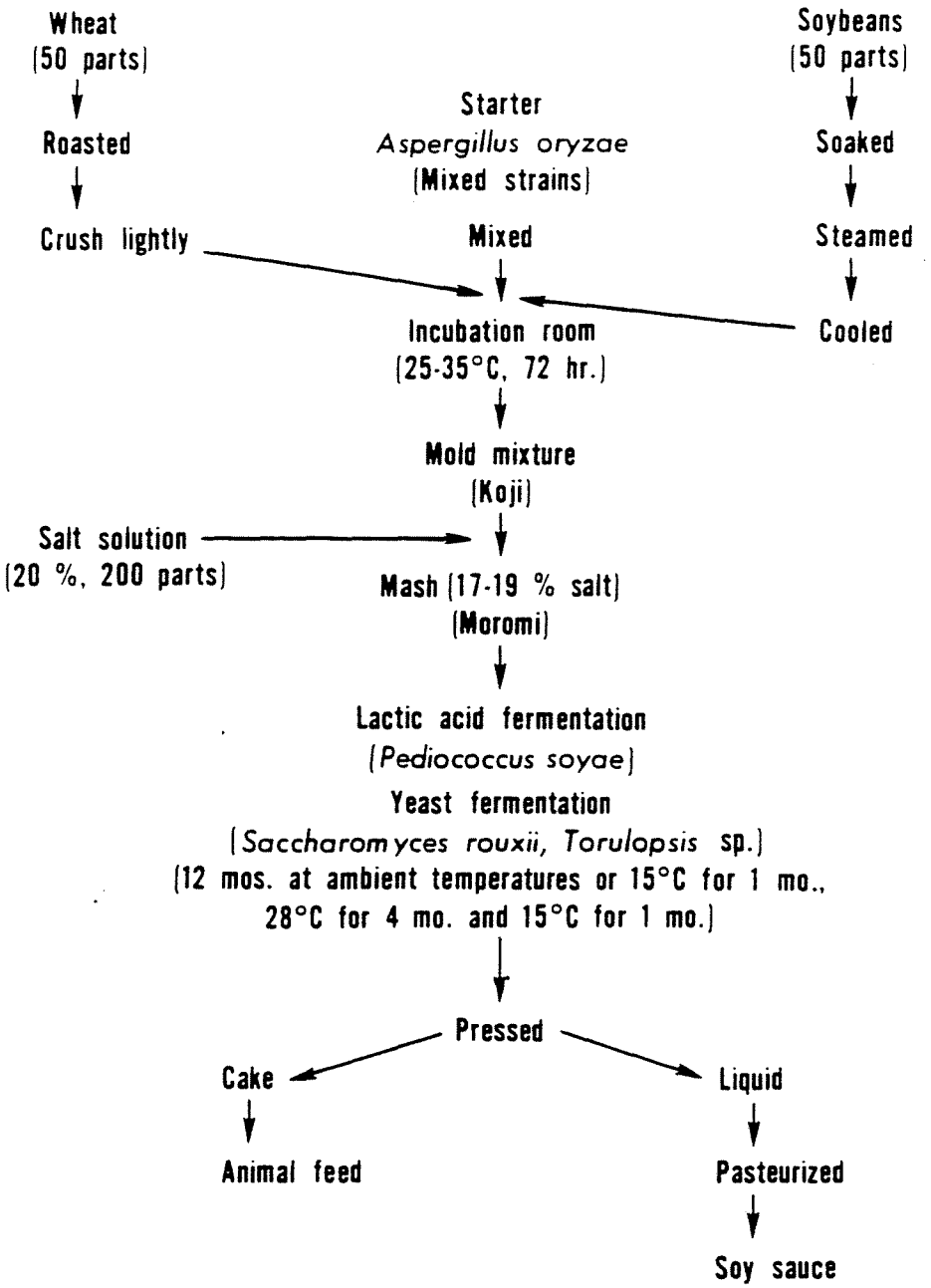

FIG. 1. Procedure for the fermentation of soy sauce (after Wang and Hesseltine, 1979).

component of the fermentation, is first roasted, then coarsely ground. Roasting adds flavor, color, and causes surface sterilization.

Equal weights of wheat and soybeans, which have been adjusted to 45% moisture, are inoculated with the spores of *Aspergillus oryzae* (Ahlb.) Cohn or *A. sojae*

TABLE I

ANALYSIS OF SULFITE LIQUOR BEFORE AND AFTER GROWTH OF *Candida utilis*[a]

Sulfite liquor fraction	g/l	
	Before growth	After growth
Hexose	10.7	0
Pentose	10.6	0
Non-assimilatable (as glucose)	4.8	5.4
Ethanol	1.3	0.6
Ether soluble (as acetic acid)	12.4	5.0
Steam distillate (as acetic acid)	16.4	11.0
Lignin	46.8	45.9
Formic acid	3.6	2.0

[a] Data from Peppler, 1970.

Sakaguchi et Yamada previously grown on steamed rice for 5 da at 30 C. The mixture is incubated in large shallow metal pans for 72 h, during which time it is periodically turned. The molded mixture, or koji, is placed in tanks with an equal volume of sodium chloride solution of sufficient density to give a final salt concentration of 17–19%. This mixture, termed moromi, is fermented for one year at ambient temperatures through the action of *Pediococcus soyae* Sakaguchi, *Saccharomyces rouxii* Boutroux, and *Torulopsis* sp. An abbreviated fermentation schedule of 1 month at 15 C, 4 month at 28 C, and a final month at 15 C seems to give equally good results. Following fermentation, the moromi is pressed to remove the raw soy sauce, which is then pasteurized at 70–80 C, treated with preservatives, and bottled.

BIOCHEMICALS

Aside from ethanol, one of the first organic compounds produced industrially using fungi was citric acid. This compound has many uses, including as an acidulent in foods, carbonated beverages, and pharmaceuticals and in the manufacture of chelating and sequestering agents as well as in plasticizers. In 1976, production was over 200,000,000 lb. Before 1919, citric acid was obtained from fruit wastes and by-products, especially from lemons, limes, and pineapples (Casida, **1968**). Many species accumulate citric acid, especially penicillia and aspergilli. Strains of *Aspergillus niger* van Tieghem are among the best microbial producers, and in

TABLE II

AMOUNT OF PROTEIN IN CONVENTIONAL PROTEIN SOURCES AND CEREALS COMPARED WITH FOOD AND FEED YEASTS

	Per cent protein
Protein sources	
Soybean meal	45
Cottonseed meal	44
Fish meal	65
Cereal	
Sorghum	11
Corn	9
Barley	12
Wheat bran	16
Yeasts	50–60

TABLE III

COMPOSITION OF CHEESE WHEY, A DAIRY WASTE WHICH MAY BE FERMENTED TO ETHANOL BY *Kluyveromyces fragilis*[a]

		Whey composition after ultrafiltration	
	Untreated whey lb	Concentrate lb	Permeate lb
Protein	6.7	6.7	–
Lactose	50.0	3.0	47.0
Ash	5.0	0.3	4.7
Other solids	3.3	0.2	3.1
Water	935	56.0	879
Total	1000	66.2	933.8

[a] Data from Pace and Goldstein, **1975**.

1919, commercial production of citric acid was begun using this species. Because of the nature of the mold, significant yields could be obtained only when the fermentation was carried out in shallow trays containing medium to a depth of 1–8 cm. This was usually an 8- to 12-da fermentation (Lowenheim and Moran, **1975**). In recent years, much of the production has been carried out in the greater convenience of deep fermentors owing to a better understanding of the factors affecting production. Even in deep fermentors, the process may take 7–8 da and is usually divided into two steps, a growth phase followed by the fermentation phase, at which time a fresh high-sugar medium is added. Regardless of whether trays or fermentors are used, strain selection is important for high yields as are nutrients and concentrations of trace metals, especially deficiencies of iron, manganese, and possibly zinc (Casida, **1968**; Demain, **1981**). Strains and conditions must also be selected to avoid production of oxalic, gluconic, malic, and 5-ketogluconic acids. Details of the process for isolating citric acid from the fermentor broth were given by Lowenheim and Moran (**1975**).

The discovery in the late 1960's that high yields of citric acid were produced by *Candida lipolytica* (Harrison) Diddens et Lodder when grown on n-paraffins (Yamada, **1977**) signaled a potential dramatic change in the fermentation industry. Yields generally exceeded 150 g/l, or about twice that synthesized by *A. niger* on sucrose. This finding occurred at a time when sugar substrates were being increasingly diverted to food use and petroleum was still inexpensive. Whether *C. lipolytica* replaces *A. niger* depends upon the price of petroleum and the concern that petroleum-derived carcinogens might be present in the final product.

Considerable additional information on industrial fermentations may be found in books by Lowenheim and Moran (**1975**) and Casida (**1968**), and in the recent review by Demain (**1981**).

TABLE IV

AVERAGE COMPOSITION FOR FARM CROP RESIDUES[a,b,c]

Cellulose	34.3
Hemicellulose	39.6
Lignin	19.9
Protein, etc.	4.6
Ash	2.5

[a] Given as dry weight per cent.
[b] Annual U.S. total for crop residues >700 million tons.
[c] Data from Tsao *et al.* (**1978**).

TREATMENT OF PROCESSING WASTES

Various agricultural and industrial processing wastes are quite high in organic material, and frequently these pollutants may be removed through the growth of appropriate microorganisms. For example, paper pulping wastes are a serious threat to lakes and streams in many areas of the U.S., partly because of the presence of D-xylose and other low molecular weight organic compounds. The ability of *Candida utilis* (Henneberg) Lodder et Kreger-van Rij to assimilate this pentose, coupled with its tolerance to lignin degradation products, makes it ideal for pulping waste treatment.

As shown in TABLE I, it is possible to remove D-xylose and other low molecular weight carbon compounds by growing *C. utilis* in mineral-supplemented waste streams. The resulting yeast cells are high in protein and suitable as a dietary supplement in foods and animal feed (TABLE II). In fact, *C. utilis* has a long history as a food yeast and was grown on wood-derived xylose in Germany during World Wars I and II (Lindner, 1922, as cited by Pyke, **1958**; Peppler, **1970**). However, one of the limitations to widespread use of yeasts as food is their high RNA content. Excessive RNA intake leads to such clinical manifestations as gout. Removal of these nucleic acids is expensive, so it is generally recommended that the intake of untreated whole yeast not exceed 25–50 g/da for adults. The shorter life span of livestock allows a much higher amount of yeast in feeds.

An additional use of *Candida utilis* has been in conjunction with *Saccharomycopsis fibuligera* (Lindner) Klöcker to dispose of starchy wastes. The basic procedure was developed by Wickerham *et al.* (**1944**) nearly 40 years ago and was put into practice in Sweden as the Symba process to dispose of potato processing wastes (Jarl, **1969**). *Saccharomycopsis fibuligera* can assimilate starch as a carbon source, but its growth rate is slow. By contrast, *C. utilis* has a rapid growth rate but cannot assimilate starch. When grown together, cross-feeding of *C. utilis* results in a substantial amount of yeast cells, which are used as an animal feed supplement.

Cheese whey represents another waste that may be fermented. Disposal frequently has been through municipal sewage systems or by spraying onto fields. The high lactose content of whey (TABLE III) has made it attractive for the production of ethanol in dairying areas. The species most commonly used for fermentation is the yeast *Kluyveromyces fragilis* (Jörgensen) van der Walt. *Saccharomyces cerevisiae* Hansen, commonly used in many other fermentations, is unable to ferment lactose. Before fermentation, the whey or whey permeate must be supplemented with nitrogen, usually in the form of ammonia, as well as with vitamins and minerals. Alcohol yields do not exceed 3–6%, making this a marginal economic process if the disposal cost of untreated whey is not taken into account. Yeast cells are recovered from the fermentation and may be used in food or animal feed. Annual production of yeast cells from this process is estimated to be around 5000 tons.

LIQUID FUEL FROM FUNGI

Because of their renewable nature, the use of farm crop residues and other biomass as a fermentation substrate for the production of liquid fuels has caught the imagination of both scientists and non-scientists. As much as 75% of crop residues may consist of potentially fermentable cellulose and hemicellulose; the remainder is primarily lignin, for which no economically feasible fermentation process has been developed (TABLE IV). Means for converting crop residue fractions into liquid fuel are depicted in FIG. 2. Fungi, because of their enzymatic

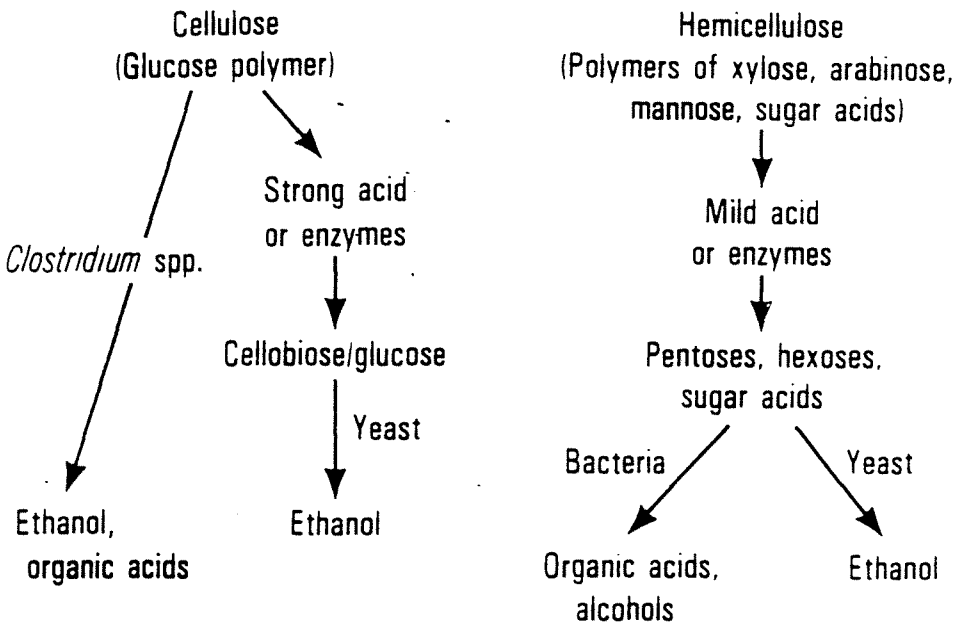

FIG. 2. Potential of cellulose and hemicellulose from crop residues for fermentation to fuel alcohol.

abilities, are of primary importance for both depolymerization and fermentation of the residues. Cellulases are reported from a number of fungi (Emert *et al.*, **1974**; Eberhart *et al.*, **1977**; Eriksson and Hamp, **1978**), but those from *Trichoderma reesei* Simmons seem to offer the greatest activity. These enzyme complexes degrade cellulose to cellobiose or glucose, which are then fermented to alcohol by yeasts. There are two main obstacles to rapid enzymatic degradation of cellulose: (1) the highly ordered crystalline structure of native fibers, and (2) the presence of lignin around the cellulose fiber (Tsao *et al.*, **1978**). Pretreatment of native cellulose with acid renders the molecule susceptible to enzymatic degradation, but lignin removal is still problematical.

The other main fraction of plant residues, hemicellulose, is relatively easily hydrolyzed to its component mono- and oligosaccharides through either mild acid treatment or appropriate enzymes (Tsao *et al.*, **1978**). Pentoses, especially D-xylose, comprise a major portion of hemicellulose, but until recently, yeasts were not known to directly ferment these five-carbon sugars, thus preventing utilization of a major fraction of plant residues for fuel alcohol. Several bacteria ferment pentoses to alcohol, but the process is relatively inefficient because of the concomitant production of short-chain organic acids (Rosenberg, **1980**; Wood, **1981**).

Recently, Schneider *et al.* (**1981**) and Slininger *et al.* (**1982**) independently discovered the yeast *Pachysolen tannophilus* Boidin et Adzet (FIG. 3) to ferment D-xylose to ethanol. This finding has now opened the way for more complete utilization of plant residues for liquid fuel. Data from Slininger *et al.* (**1982**) show *P. tannophilus* to yield 0.34 g of ethanol per gram of D-xylose consumed. Oxygen is required for cell growth, but not for alcohol production. Ethanol tolerance of

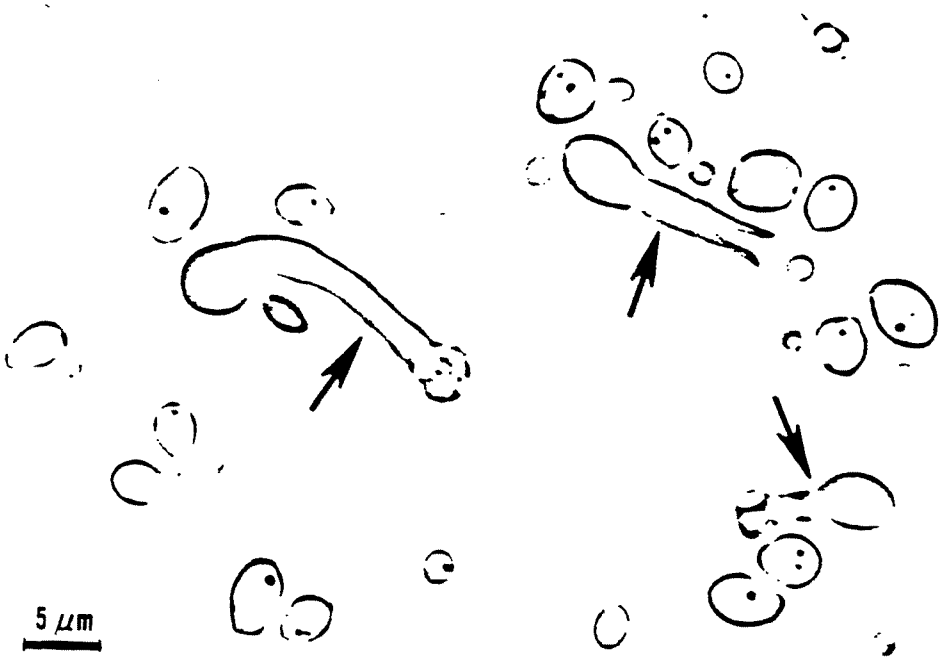

FIG. 3. *Pachysolen tannophilus* NRRL Y-2460. Asci have tubelike ascophores (arrows). Left ascophore shows conjugant at the base, whereas the other two ascophores are unconjugated and were derived from diploid cells. Asci form within the V-shaped notch of the ascophore and produce 4 hat-shaped ascospores. Vegetative cells, some with one or two buds, are also present.

this yeast is somewhat low at 3%, but it is anticipated that higher levels may be obtained through strain selection and modification.

FUTURE PROSPECTS

The successes of past utilization of fungi to meet human needs coupled with an expanding world population would mandate an increased future reliance upon fungi for the biosynthesis of useful compounds. Fermentations utilizing organic wastes and low value biomass may appear much more economically feasible then than they do now.

The key to future successes will rest on a number of factors: innovative uses of presently known species, discovery of useful new species, and genetic modification of known as well as yet to be discovered fungal germplasm. Recent examples of new uses for known species may be found in the application of *Cyathus stercoreus* (Schw.) de Toni to removal of lignin during fermentation of plant residues (Wicklow *et al.*, **1980**) and the fermentation of D-xylose from hemicellulose directly to ethanol by *Pachysolen tannophilus* (Schneider *et al.*, **1981**; Slininger *et al.*, **1982**).

Despite the potential of natural isolates, major advances in the utilization of fungi for biosynthetic activities will probably come from genetically modified strains. Conventional breeding programs are important and frequently worth the

resources invested. However, considerable progress may be expected through methods classified under the term "genetic engineering." Techniques include transformation, protoplast fusion and the introduction of specifically cloned genes into host cells through plasmid vector systems.

By use of transformation, Russell and Stewart (**1980**) successfully transferred the ability to ferment maltotriose from one yeast to another. The technique involved incubation of spheroplasts from the recipient strain with DNA from the donor strain. The spheroplasts were then transferred to a medium appropriate for cell wall regeneration. Among the treated cells, one was found capable of maltotriose fermentation, suggesting DNA uptake and incorporation into the recipient strain genome. However, transformation carried out in this manner is based on chance and is rather inefficient.

Protoplast fusion offers yet another means for combining the genetic characteristics of two strains. Cells are enzymatically stripped of their walls and placed in contact to allow fusion. Various stabilizing buffers and agents to promote fusion have been developed (Ferenczy, **1981**). The advantage of this technique is the combination of characteristics not likely to be achieved through breeding. This is especially true when fusions have been made between different species or between strains of disparate ploidy. It is also in this technique that the taxonomist can have significant impact. Fusions are more likely between closely related than distantly related taxa. An understanding of species relationships can enhance the likelihood of selecting potentially successful fusion partners.

Genetic modification through introduction of specific genes or groups of genes became a reality with the discovery of a yeast plasmid vector system (Beggs, **1978**; Hinnen *et al.*, **1978**). Genes may be isolated, cloned, enzymatically incorporated into an appropriate vector such as a plasmid, and allowed to enter the recipient cell. The introduced genes may then replicate as the plasmid replicates or the genes may actually integrate into a chromosome.

The success of recombinant DNA technology is dependent not only upon developing suitable vectors but also upon two additional requirements: identifying and cloning all genes in the pathway of interest, a potential difficulty, since the genes may not be colinear on the chromosome, and the development of an understanding of the control mechanisms that allow expression of the recombinant genes in their new host environment. The potential of recombinant DNA technology is enormous, and it can be expected that fungi will have a major impact both as gene donors and as recipients of modified genes.

Key Words: fermented foods, process waste fermentation, biomass fermentation, D-xylose fermentation.

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